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Aqueous Solutions that Model the Cytosol: Studies on Polarity, Chemical Reactivity and Enzyme Kinetics.

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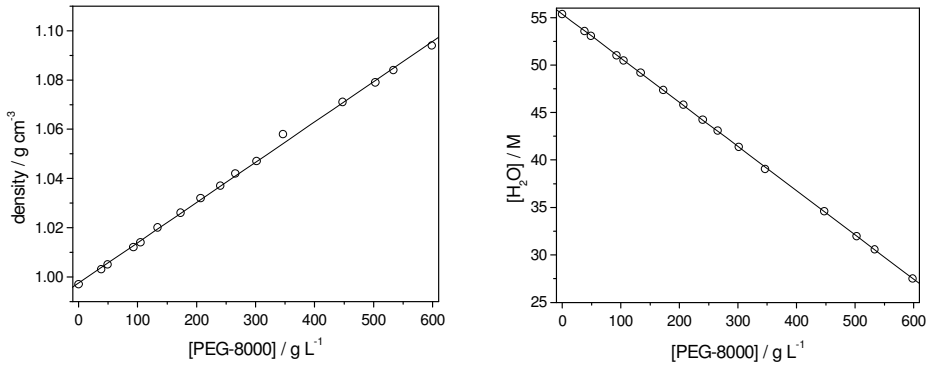
Variation of density with solute concentration



Variation of water concentration and density with PEG concentration in binary mixtures.

Solutions of Reichardt's dye in common solvents and amide additives

Note – the blue flecks in the water sample are undissolved $E_T(30)$ probe.

Discussion of possible general base catalysis by amide solutes

There is insufficient data for a definitive analysis, but it appears that at low water concentrations, general base catalysis by the amide solute becomes important. A Brønsted analysis assuming only general base catalysis gives the following rate equation for the reaction of substrate ***S*** in the presence of general base ***B*** (Eqn. 3) where k''' is the third order rate constant for the water catalysed reaction, β is the Brønsted co-efficient for the base catalysed reaction and ΔpKa is the difference between the pK_as of water and the general base:

Rate law for termolecular ester hydrolysis derived from Brønsted analysis.

$$\frac{d[S]}{dt} = -k'''.[S].[H_2O].\left\{[H_2O] + 10^{\beta.\Delta pKa}.[B]\right\}$$

Some simple amides, such as DMA, may be stronger bases than water by as much as 2 pK_a units,[1] but assuming a value of 0.4 for β and factoring in concentration, this catalysis will not be significant until the water concentration drops below 10 M. This analysis does not take account of direct nucleophilic attack by only one water molecule, nor the possibility of nucleophilic catalysis by the solute, which potentially adds two further rate constants and concentration terms to the observed rate equation. The picture is further complicated by the possibility that the pK_as of all species present are almost certain to vary with the composition of the solution and that the rate constant (k''') and Brønsted co-efficient (β) determined in pure water are unlikely to be constant throughout the whole solute concentration range studied.

[1] Bagno A.; Lovato, G.; Scorrano, G. J. Chem. Soc., *Perkin Trans. 2* 1993, 1091-1098

Tables of experimental data

Table 1: Variation of water concentration and density with PEG-8000 concentration.

[PEG] / g L^{-1}	[H_2O] / M	Density / g cm^{-3}
0.0	55.34	0.997
38.3	53.55	1.003
49.1	53.06	1.005
92.9	51.02	1.012
104.8	50.47	1.014
134.0	49.18	1.020
172.8	47.36	1.026
206.8	45.80	1.032
240.1	44.24	1.037
265.8	43.09	1.042
301.6	41.38	1.047
346.6	39.05	1.058
447.6	34.60	1.071
502.9	31.98	1.079
533.5	30.56	1.084
598.6	27.50	1.094

Table 2: Variation of $E_T(30)$ with concentration of simple solutes.

	[Solute] / M	[H_2O] / M	$E_T(30)$ / kcal mol^{-1}
Water	0.000	55.34	62.71
Urea	1.332	52.01	62.45
	3.401	46.95	61.43
	6.806	38.37	60.89
	7.609	36.30	60.45
	9.097	32.51	60.27
Sorbitol	0.159	54.29	63.05
	0.323	53.18	62.92
	0.507	51.93	62.64
	0.696	50.69	62.74
	1.126	47.78	62.96
	1.576	44.85	62.95
	2.304	39.87	62.66
	2.425	38.98	62.31
	3.059	34.95	62.04
Formamide	1.759	51.66	61.90
	1.820	51.51	62.44
	3.834	47.37	61.52
	5.772	43.27	61.24
	6.960	40.43	60.66
	9.403	35.30	60.09
DMA	0.032	47.88	59.91
	3.111	40.33	57.95
	4.211	35.00	56.74
	5.828	27.27	55.08
	6.978	21.54	53.22

Table 3: Variation of $E_T(30)$ with concentration of PEGs.

	[PEG] / g L^{-1}	[H_2O] / M	$E_T(30)$ / kcal mol^{-1}
Water	0.0	55.34	62.71
PEG-200	211.5	45.55	60.44
	390.6	36.94	57.97
	568.9	28.32	56.30
	616.8	26.04	55.59
	649.4	24.46	55.23
PEG-400	51.0	52.90	61.41
	78.1	51.78	61.18
	214.4	45.27	58.89
	318.0	40.47	57.77
	505.8	31.65	55.75
	684.0	23.102	53.60
	829.2	15.695	51.98
PEG-1500	33.2	53.83	61.71
	79.6	51.65	60.71
	155.8	48.08	59.25
	254.5	43.55	58.4
	297.1	41.57	57.94
	397.4	36.89	56.54
	447.6	34.6	55.58
	586.3	27.96	53.78
PEG-8000	38.3	53.55	61.16
	104.8	50.47	59.97
	307.4	41.06	57.63
	502.9	31.98	55.04
	533.5	30.56	54.32
	598.6	27.5	53.43

Table 4: Variation of $E_T(30)$ with concentration of N-CHP.

[N-CHP] / M	[H_2O] / M	$E_T(30)$ / kcal mol^{-1}
0.000	55.34	62.71
0.094	54.58	61.83
0.111	54.42	61.42
0.118	54.36	61.13
0.161	53.91	60.89
0.216	53.28	59.77
0.259	53.11	58.96
0.379	52.04	56.19
0.436	51.52	55.62
0.505	50.93	55.76
0.544	50.63	55.32
0.689	49.33	54.31
0.908	47.42	54.00
1.052	46.13	53.70
1.767	39.77	52.81
2.526	33.01	52.40
3.067	28.31	52.34

Table 5: $E_T(30)$ probe-BSA binding experiment.

10^6.[BSA] / M	$E_T(30)$ / kcal mol^{-1}
0.00	62.75
0.00	62.56
0.00	62.51
0.00	62.48
0.00	62.45
0.61	62.12
1.52	61.52
3.04	60.00
3.05	59.57
3.66	60.73
4.26	59.06
4.88	58.20
5.47	58.05
6.10	57.17
6.10	57.15
8.51	56.75
8.54	57.05
9.15	56.12
11.55	55.99
12.20	55.90
13.42	55.84
13.42	55.76
14.59	56.06
18.30	55.65
19.52	55.49
19.52	55.15
20.74	55.26
24.40	55.61
26.84	55.15
29.89	55.14
31.11	55.45
35.38	55.42
35.38	55.18
36.60	55.40
36.60	55.23
42.70	55.36
42.70	55.21
45.75	55.21
49.41	55.40
54.90	55.42
61.01	55.37
61.01	55.24
61.01	55.16

Table 6: $E_T(30)$ probe-BSA binding Job-plot data.

$\delta E_T(30)$.mol.frac.$(BSA)^{-1}$ / kcal mol^{-1}	mol.frac.(BSA)
0.18	0.952
0.30	0.380
0.39	0.097
0.47	0.019
0.53	0.087
0.57	0.121

Table 7: Rate data for the neutral hydrolysis of 4-nitrophenyl dichloroacetate in the presence of amides.

	$[H_2O]$ / M	[amide] / M	k_{obs} / s^{-1}	log (k_{obs} / s^{-1})
water	55.33	0.000	0.0594	-1.226
DMA	53.48	0.378	0.0448	-1.348
	52.47	0.580	0.0479	-1.320
	52.19	0.638	0.0456	-1.341
	50.37	1.022	0.0390	-1.409
	46.82	1.738	0.0358	-1.446
	43.89	2.330	0.0324	-1.489
	41.52	2.851	0.0297	-1.528
	38.36	3.470	0.0263	-1.580
	34.91	4.169	0.0234	-1.631
	23.01	6.687	0.0118	-1.926
	15.32	8.161	0.0072	-2.144
	12.87	8.622	0.0046	-2.341
N-CHP	54.49	0.104	0.0476	-1.322
	54.27	0.121	0.0502	-1.299
	53.24	0.245	0.0424	-1.373
	52.43	0.338	0.0360	-1.444
	52.06	0.378	0.0310	-1.509
	50.65	0.541	0.0188	-1.726
	49.76	0.643	0.0158	-1.801
	44.85	1.196	0.0112	-1.951
	40.32	1.714	0.0096	-2.016
	35.86	2.212	0.0097	-2.015

Table 8: Rate data for the neutral hydrolysis of 4-nitrophenyl dichloroacetate in D_2O the presence *N*,*N*-dimethylacetamide.

	$[D_2O]$ / M	[DMA] / M	k_{obs} / s^{-1}	log (k_{obs} / s^{-1})
D_2O	55.17	0.000	0.0203	-1.693
DMA	47.80	1.496	0.0131	-1.883
	46.90	1.702	0.0125	-1.902
	37.61	3.628	0.0077	-2.112

Table 9: Observed rate constant data for the TRIS-catalysed hydrolysis of 4-nitrophenyl acetate in the presence of PEG-400.

[TRIS] / M	$10^5.k_{obs}$ / s^{-1}				
	0 g L^{-1} PEG	87 g L^{-1} PEG	171 g L^{-1} PEG	260 g L^{-1} PEG	341 g L^{-1} PEG
0.04	1.46	1.49	1.51	1.64	1.60
0.2	4.96	4.87	4.66	4.38	3.93
0.3	7.46	7.05	6.70	6.24	6.05
0.4	9.42	8.99	8.63	7.85	6.99
0.5	11.9	11.4	10.2	9.63	8.70
0.6	14.3	13.4	12.2	11.2	10.3

Table 10: Second order rate constants for the TRIS-catalysed hydrolysis of 4-nitrophenyl acetate in the presence of PEG-400.

$[H_2O]$ / M	$10^4.k''$ / $M^{-1}s^{-1}$	log (k'' / $M^{-1}s^{-1}$)
55.3	2.28	-3.64
51.4	2.14	-3.67
47.3	1.90	-3.72
43.0	1.72	-3.76
38.9	1.54	-3.81

Table 11: Observed rate constant data for the specific acid-catalysed hydrolysis of 2-(4-nitrophenyl) tetrahydropyran in the presence of PEG-400.

[HCl] / M	$10^3.k_{obs}$ / s^{-1}				
	0 g L^{-1} PEG	108 g L^{-1} PEG	215 g L^{-1} PEG	322 g L^{-1} PEG	431 g L^{-1} PEG
0.01	1.82	1.32	0.89	0.45	0.28
0.02	2.90	2.53	1.75	1.13	1.02
0.05	6.97	5.62		3.52	2.30
0.058			5.02		
0.07	9.67	7.34	5.91	4.52	3.34
0.10	13.6	10.4	8.66	7.22	5.17

Table 12: Second order rate constants for the specific acid-catalysed hydrolysis of 2-(4-nitrophenyl) tetrahydropyran in the presence of PEG-400.

$[H_2O]$ / M	k" / $M^{-1}s^{-1}$	log (k" / $M^{-1}s^{-1}$)
55.3	0.132	-0.879
50.4	0.100	-1.00
45.3	0.086	-1.07
40.1	0.074	-1.13
34.9	0.052	-1.28

Table 13: Rate data for the Diels-Alder reaction between 1,4-naphthoquinone and cyclopentadiene in the presence of PEG-400.

$[H_2O]$ / M	k'' / $M^{-1}s^{-1}$	log (k'' / $M^{-1}s^{-1}$)
55.3	5.58	0.747
50.1	4.47	0.651
47.4	4.53	0.656
42.7	3.81	0.581
53.7	4.81	0.682
51.8	5.17	0.713
44.5	4.29	0.632
40.5	4.19	0.622
37.4	3.67	0.564
31.8	2.38	0.377
34.7	2.85	0.454
30.4	1.87	0.273
27.7	1.69	0.228

Table 14: Variation of Michaelis-Menten parameters for the trypsin-catalysed hydrolysis of 4-nitrophenyl acetate upon addition of poly(ethylene glycol), *N-tert*-butyl acetoacetamide and urea.

	$[H_2O]$ / M	$10^3\ k_{cat}$ / s^{-1}	$10^3\ K_M$ / M
PEG-8000	55.07	2.78	0.50
	49.78	2.35	0.55
	43.73	1.80	0.54
	36.86	1.06	0.56
	52.85	2.56	0.53
	48.89	2.15	0.55
	46.18	1.97	0.56
	39.05	1.35	0.59
	41.21	1.44	0.54
NBAA	54.97	2.78	0.38
	43.09	1.21	0.66
	48.22	1.96	0.56
	52.34	2.48	0.47
	40.59	0.86	0.64
Urea	55.08	2.77	0.39
	51.22	3.06	0.75
	49.4	3.26	0.97
	53.22	3.08	0.61
	55.08	2.79	0.44
	40.58	3.58	1.80
	42.88	3.54	1.58